AMENDMENT UNDER 37 C.F.R. § 1.111 Attorney Docket No.: Q95279

Application No.: 10/581,911

**AMENDMENTS TO THE CLAIMS** 

This listing of claims will replace all prior versions and listings of claims in the

application:

LISTING OF CLAIMS:

1. (currently amended): A method for making cartilage tissue which comprises:

(a) two-dimensionally culturing bone marrow mesenchymal stem cells to confluence-;

(b) subculturing the cells from the confluent two-dimensional culture to-before three-

dimensional culture;

(c) three-dimensionally culturing the cells of step (b) without carrier in a simulated

microgravity environment with the use of a uniaxial rotary bioreactor that realizes a simulated

microgravity environment on the earth by compensating the ground gravity with the stress

resulting from controlled rotation speed, in the presence of an inducer of cartilage differentiation;

and

(d) obtaining cartilage tissue expressing Type II collagen.

2. (original): The method according to claim 1, wherein the simulated microgravity

environment provides gravity that is 1/10 to 1/100 of the ground gravity to an object on a time-

average basis.

3.

(previously presented): The method according to claim 1, wherein the simulated

microgravity environment provides gravity that is 1/10 to 1/100 of the ground gravity to an

object by compensating the ground gravity with the stress resulting from controlled rotation

speed.

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4. (canceled).

5. (previously presented): The method according to claim 1, wherein the bioreactor that realizes a simulated microgravity environment on the ground is a Rotating Wall Vessel (RWV) bioreactor.

- 6. (previously presented): The method according to claim 5, wherein culture is conducted by seeding bone marrow cells at a density of 10<sup>6</sup> to 10<sup>7</sup> cells/cm<sup>3</sup> at a rotation speed of 8.5 to 25 rpm when a 5 cm in diameter RWV vessel is used.
- 7. (previously presented): The method according to claim 1, wherein culture is conducted by adding TGF-β and/or dexamethasone to a culture medium.
  - 8. (canceled).
- 9. (previously presented): The method according to claim 1, wherein the bone marrow mesenchymal cells are isolated from a subject in need of transplantation of the cartilage tissue.
- 10. (previously presented): The method according to claim 1, wherein the cartilage tissue has a major axis of 1 cm or more.

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11. (previously presented): The method according to claim 1, wherein the rotation speed is adjusted to synchronize with the sinking speed of the seeded cells to minimize the influence of the ground gravity on the cells.